corresponding to the peptide A binding site. We tested the effect of beta scorpion toxins - having a similar structure - on the RyR1 function. Charibdotoxin (CHTX) elicits close state at 20 nM in an all or none and voltage dependent manner because of smaller surface charge. Smaller size makes it easier to reach the most inner toxin binding site (out of the three) which causes the closure of the channel. MCa and CHTX share a common binding site which is identical to the peptide A binding site. Noxiustoxin has a similar effect at slightly higher toxin concentration. At nanomolar concentration Kaliotoxin evokes ''flickering'' of the channel in subconductance state which is occasionally interrupted by long lasting closed states, while locks the channel in closed state at micromolar concentration. Iberiotoxin induces a slight increase of the open probability accompanied by normal gating while Slotoxin has no effect. With the exception of MCa all toxins are effective only at one side, at the preferred side. Iberiotoxin and Slotoxin - ion spite of similar structure - have no large positive surfaces, they exhibit random surface charge distribution. A model has been proposed for the possible mode of action which accounts for the above effect of the tested toxins. Supported by Hungarian Research Found OTKA 81923.

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Modulation of Ryanodine Receptor Channels (RyRs) by Penaresin and Eudistomin Derivatives

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Penaresins and eudistomins are bioactive metabolites from marine organisms that were found to induce Ca^{2+} release from skeletal muscle sarcoplasmic reticulum (SkMSR). As these compounds could have characteristics of supercaffeines, we have carried out studies to contrast their ability to modulate RyRs versus that of caffeine. Two eudistomin D derivatives were tested: bromoeudistomin D (BED) and its putatively more potent analog N-methylbromoeudistomin D (MBED). We also tested penaresin (PE) and four PE analogs: N-methyl-PE (MPE), nitro-PE (NPE), dicyano-PE (DCPE), and N-methyl-PE-methylester (MPEM). We utilized rabbit SkMSR microsomes to evaluate the effect of penaresins and eudistomins on RyRs by measuring a) rates of Ca^{2+} loading and Ca^{2+} release and b) [3H] ryanodine binding. We also tested the effects of all the compounds on the activity of sarcoplasmic reticulum Ca^{2+} ATP-ase (SERCA). We found that BED and MBED mimic caffeine in the capacity to activate RyRs. At the doses required to fully activate RyRs, BED also inhibited SERCA while MBED did not affect it. In our hands, penaresins were weaker RyRs agonists and only partially matched the action of caffeine (at very high concentrations, close to the drugs limit of solubility). Regarding the effects on SERCA activity, PE was inhibitory while MPEM displayed apparent agonistic action. MBED, the most potent RyRs agonist, mimicked the reversible action of caffeine on the open probability and gating kinetics of RyRs reconstituted into planar lipid bilayers. Confocal microscopy studies are underway to compare the ability of these compounds versus caffeine to induce SR calcium release in myocytes. In principle, our studies indicate that eudistomins (especially MBED), but not penaresins, are potent ''caffeine-like'' agonists of RyRs (supported by NIH RO1 GMO78665).

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Downstream Effects of a RyR1 Mutation Linked to Malignant Hyperthermia on the Phenotypic and Functional Characteristic of Dendritic Cells

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The skeletal muscle ryanodine receptor 1 (RyR1) isoform is not only expressed in skeletal muscle, but also in immune cells, particularly in B lymphocytes and dendritic cells where it is involved in maturation and antigen presentation. This implies that mutations causing gain of function of RyR1 may have an effect not only on skeletal muscle but also on the signaling event leading to activation of immune response via antigen presenting cells expressing RyR1. We investigated the phenotype and function of dendritic cells obtained from the Y522S *RYR1* knock in mouse, an animal model carrying a mutation linked to Malignant Hyperthermia. We show that CD11c ''+ve'' cells isolated from spleens of WT and HET $RYR1_{Y522S}$ knock in mice express RyR1 and that there is a significant increase in the surface expression of the maturation marker CD83 in MHCII positive dendritic cells from $RYR1_{Y522S}$ mice compared to their wild type littermates. The increase in dendritic cell maturation markers was linked to a significant increase in the % CD4 +ve T helper cells in the splenic cell population. We also investigated whether the presence of the *RYR1* mutation affects the capacity of dendritic cells to activate T-cells. Mixed lymphocyte reactions between dendritic cells from WT or $RYR1_{Y522S}$ mice with the Bl6 background and T-cells isolated from Balb-c mice show that dendritic cells carrying the Y522S *RYR1* mutation have an increased capacity to stimulate T cells. Based on the present results we suggest that the presence of *RYR1* mutations will not only affect muscles, but will also affect the functional properties of the immune system.

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Coupled Gating of Skeletal Ryanodine Receptor Channels - a Summary of Two Decades of Studies in Planar Lipid Bilayers

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When reconstituted into planar lipid bilayers, ryanodine receptors (RyRs) from skeletal muscle sarcoplasmic reticulum (SR) display coupled gating, i.e. synchronous opening/closures. Coupled gating was observed in ~20% of reconstitutions after ATP/Mg2+ were added to the cytosolic solution. In the absence of ATP/Mg2+ coupled gating was not evident in 20-90 min recordings from hundreds of multichannel reconstitutions. ATP can be partially replaced by other nucleotides (e.g. ADP, AMP) and does not appear to exert its modulatory action through RyR1 phosphorylation. Agent that affect cytoskeleton did not impair coupling. Luminal Ca2+ was also found to be crucial for finding coupled gating. Although a hypothetical participation of local Ca2+-induced Ca2+ release in the coupling phenomenon cannot completely be ruled out, direct luminal effects of Ca2+ on RyR1 seem to be key for RyR1 coupling. Coupled gating of RyR1 was not affected by FKBP12 addition or removal (by long term exposure to rapamycin). In some recordings, periods of coupled gating (seconds to minutes) are intercalated with periods of uncoupled events suggesting that reversible changes in the gating status of interacting RyRs could be important for coupling. In this regard, coupled gating was triggered by agonists (caffeine, menthol and halothane) and was impaired by high Mg2+ or tetracaine, all know to affect RyR gating status. We believe that coupled gating results from physical interactions (possibly of electrostatic nature) between two classes of RyRs. This heterogeneity of RyRs is reflected both in their gating and conductance. In summary, in the cellular environment coupling would be highly dependent on ATP/Mg2+ levels and SR Ca2+ load (supported by NIH RO1 GMO78665).

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Arrhythmogenic NCX Currents in Cardiomyocytes from Transgenic Mice Expressing Mutated RyR2 Receptors

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We investigated Ca2+ signaling in cardiomyocytes from transgenic mice with a mutation in the calmodulin (CaM) binding domain of RyR2 (W3587A/L3591D/F3603A, RyR2ADA; Yamaguchi et al., Circ Res, 100:293-5, 2007). Ca2+ sparks (Fluo-4) in cardiomyocytes from 11-15 day old homozygous mutant mice were less frequent (0.26 ± 0.17 vs. 3.62 ± 0.54 sparks/sec n=18), but longer lasting than in wild-type. Voltage-clamp depolarization of the mutant cells produced Ca2+ transients of reduced amplitude even though those triggered by caffeine were enhanced and accompanied by disproportionally larger Na+-Ca2+ exchanger (NCX) currents. The distributions of RyR2, NCX and membrane staining in mutant cells indicate a disorganized t-tubular system. During repeated voltage-clamp depolarizations, mutant cardiomyocytes produced alternans or gradually rising Ca2+ transients that in time developed a tendency to generate large delayed Ca2+ releases and NCX currents thereby restarting the sequence. Our findings suggest that the expression RyR2ADA in homozygous mice with cardiac hypertrophy is associated with impaired ICa-induced Ca2+ release resulting, In part, from disruption of the t-tubular system and its associated dyadic junctions. At the same time it is noticeable that the (partially decoupled) SR Ca2+ stores are well maintained and produce a) infrequent, but long lasting Ca2+ sparks, and b) large regenerative Ca2+ transients that are accompanied by prominent NCX currents and can be triggered, not only by caffeine, but also by repeated stimulation. The latter observation suggest a mechanism for delayed afterdepolarization, where the arrhythmogenic potential of NCX currents is enhanced by the concurrence of a number of factors: a) impaired Ca2+ signaling by RyR2, b) partial structural decoupling of components of the SR Ca2+ stores, and c) gradual enhancement of the Ca2+ contents of these stores resulting e.g. from rapid beating during adrenergic stimulation. (NIH HL16152; AHA 10SDG3500001).